WR

AMERICAN JOURNAL OF PHARMACY

and

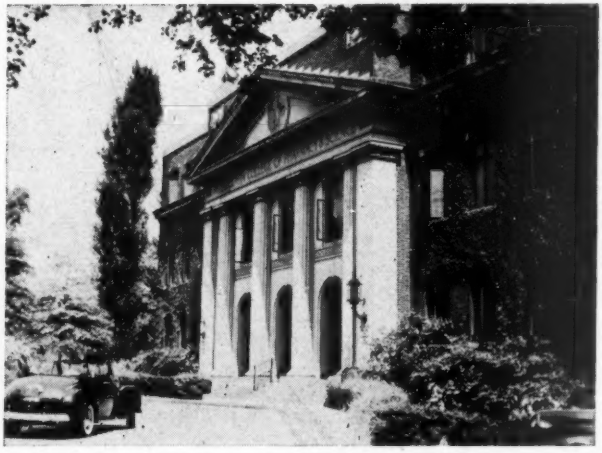
THE SCIENCES SUPPORTING PUBLIC HEALTH

PUBLIC LIBRARY
OCT 6 1944
DETROIT

SINCE
1825

DR. HOWARD WALTER FLOREY
To him and American Industry we owe a debt of gratitude for the present status of penicillin

AUGUST
1944



New Classes

In

PHARMACY
CHEMISTRY
BACTERIOLOGY
BIOLOGY

COMMENCING

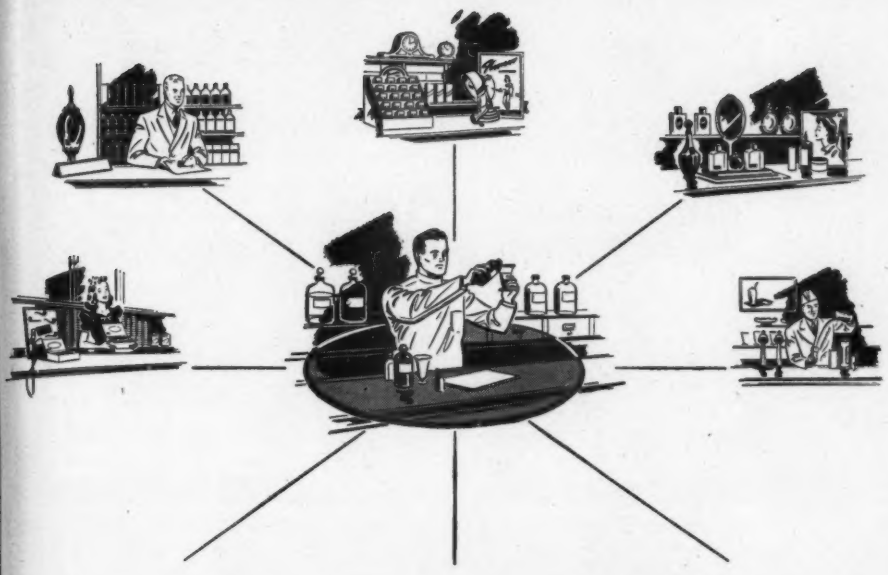
OCTOBER 30

1944

PHILADELPHIA COLLEGE OF PHARMACY AND SCIENCE

Founded in 1821, and Located at Forty-third Street, Kingsessing and Woodland Avenues, Philadelphia 4, Pa.

Write for Catalog



A PROFESSIONAL RESPONSIBILITY

The first apothecary shop was established back in the thirteenth century for the purpose of compounding and dispensing the medicines which physicians prescribed. From that day to this, the prescription department has been the distinguishing feature of the drug store professionally, its safeguard against losses economically. It is the hub around which the entire drug industry revolves. Without the prescription department, the drug store as we know it would soon cease to exist.

Every pharmacist should assure himself that his prescription department is adequate to modern demands, that its operation is governed by sound business methods, that ethical principles are observed. In order to benefit from the opportunities which the profession affords, the pharmacist must also accept its responsibilities. In the selection of stocks he can do no better than to concentrate on the Lilly Line. Lilly Products are high in quality and find ready acceptance with physicians.

ELI LILLY AND COMPANY

INDIANAPOLIS 6, INDIANA, U. S. A.

You won't find PARKE-DAVIS vitamin products in a bin at the supermarket, next to the soap counter at the corner grocery, or in with the cough drops at the newsstand.

FOUR FACTS

1. The P-D vitamin line is complete . . . there is a product that meets the particular needs of every vitamin deficiency.
2. The P-D vitamin line is detailed and advertised to physicians.
3. The P-D line is backed by a name that customers know and respect.
4. The P-D vitamin line is a "drug store only" line.

We believe that vitamins will continue to occupy an increasingly important place in the health and welfare of the nation . . . that they should be taken on the advice or prescription of a physician and should be sold only by pharmacists who understand them.

That is why PARKE-DAVIS vitamin products are *professionally distributed* . . . detailed and advertised to physicians . . . sold only through the drug trade.

You can *hold the vitamin line* if you display and feature the PARKE-DAVIS label as the line of professional preference. It brings customers back to your pharmacy for repeat sales.

Parke, Davis & Company

DETROIT 32, MICHIGAN

ACME

The medical soldier— **does he carry weapons?**

He might be a pharmacist from Waukegan or New York, from Stroudsburg or San Francisco. He wears the Caduceus—the emblem with serpents twined around the winged staff of Mercury. In this war, as in other wars, he carries no firearms. But, he certainly carries weapons: the fine ideal of service above self, excellent knowledge of war medicine and the skill to apply it. He is tough and tender—fighting mad, yet calm enough in the thickest of battle to safeguard the lives of his buddies.

In combat, he moves with the troops or parachutes to wounded men, works 60 hours at a stretch, is active under constant bombardment.

He is the "Medical Soldier" serving in the front line of battle. He reaches the wounded promptly, often in seconds, and gives expert emergency treatment.

They say the Medical Soldier carries no weapons. No? Into the thick of battle he carries a staunch heart, unbounded courage, enviable skill, and the devoted desire to give his all, if necessary, to reclaim and perpetuate liberty.

A tribute to the members of the

U. S. ARMY MEDICAL DEPARTMENT

ACME

He is the litter-bearer or human ambulance of the front line. He carries wounded to Battalion Aid Stations, the "hospitals" that move forward each time our forces advance into enemy territory.

ATABRINE • Spinal anesthetics • Sulfa drugs
PENICILLIN • DIODRAST • SALYRGAN-THEOPHYLLINE
are only a few of the Winthrop products which supplement the work of the Medical Soldier.

WINTHROP CHEMICAL COMPANY, Inc.
Pharmaceuticals of merit for the physician

when apathy prolongs convalescence

Many convalescent patients, faced with the "drab succession of dreary days", may develop a reactive depression which can markedly retard normal recovery.

This depression may manifest itself in symptoms of apathy, hopelessness or despondency, psychomotor retardation and subjective weakness.

Obviously, the physician should guard against undue stimulation. But when, in his judgment, a convalescent patient will benefit by a sense of increased energy, mental alertness and capacity for work, the administration of Benzedrine Sulfate Tablets will often accomplish the desired result.

BENZEDRINE
SULFATE TABLETS
(racemic amphetamine sulfate)

As with any potent therapeutic agent, Benzedrine Sulfate should be administered under the supervision of the physician. Indications and contraindications are set forth in N.N.R.

SMITH, KLINE & FRENCH LABORATORIES • PHILADELPHIA, PA.

AMERICAN JOURNAL OF PHARMACY

AND THE SCIENCES SUPPORTING PUBLIC HEALTH

Since 1825

LINWOOD F. TICE, Ph. G., B. Sc., M. Sc., Editor

Austin A. Dodge, Ph. C., B. Sc., Ph. D., Assistant to the Editor

John E. Kramer, B. Sc., Business Manager

COMMITTEE ON PUBLICATION

E. Fullerton Cook, P. D., Ph. M., M. S., Chairman

Mitchell Bernstein, P. D., M. D., F. A. C. P. — Louis Gershenfeld, P. D., Ph. M., D. Sc.

Marin S. Dunn, A. M., Ph. D. — John K. Thum, Ph. G., Ph. M.

J. W. Sturmer, Phar. D., Ph. M., D. Sc., Secretary

Ivor Griffith, P. D., Ph. M., D. Sc., F. R. S. A., ex officio

Vol. 116. AUGUST, 1944 No. 8

CONTENTS

PAGE

Editorial:

The Responsibility of the Pharmacist in the Sale of Vitamins 288

Articles:

Progesterone, a Hormone of the Corpus Luteum. By Willard M. Allen 291

Estimation of Total H_2S Metals in Iron Salts. By H. Rosenblum and Jos. Rosin 296

A Preliminary Report on a Simplified Technic of the Ruge Virulence Test With Some Clinical Observations. By L. Gershenfeld and M. R. Cohen 301

Selected Abstracts 310

Solid Extracts 313

Book Reviews 315

Published monthly by the Philadelphia College of Pharmacy and Science
43rd Street, Kingsessing and Woodland Avenues, Philadelphia 4, Pa.

Annual Subscription, $3.00 — **Foreign Postage, 25 Cents Extra**

Single Numbers, 30 Cents — **Back Numbers, 50 Cents**

Entered as Second-Class Matter at the Post Office at Philadelphia, Pa. Under Act of March 3, 1879

Acceptance for Mailing at Special Rate of Postage Provided for in Section 1103 Act of October 3, 1917. Authorized February 15, 1920

E D I T O R I A L

THE RESPONSIBILITY OF THE PHARMACIST IN THE SALE OF VITAMINS

IN several states legislation has already been passed restricting the sale of certain vitamin products to registered pharmacies. In a number of other states similar legislation is in process. The arguments that have been advanced in order to accomplish this legislation are sound and we shall briefly survey a few of the more pertinent ones. In the first place vitamins are undoubtedly classifiable as drugs, at least in the form that they are offered for sale to the public. Foods do, of course, contain all the essential vitamins necessary for a *normal individual eating a well-balanced diet,* but such optimum conditions are probably not found in large numbers of the people even in the so-called higher income brackets.

Many persons, due to certain disturbances in metabolism or assimilation, either fail to utilize those vitamins obtained in food or require a larger supply than a normal diet provides. Others have fixed dietary habits resulting in a deficiency of one or more of the necessary factors which, if the deficiency is not pronounced, may produce only subclinical symptoms. Thus we have a wide variety of conditions due to a lowered vitamin supply ranging from the severe hemorrhagic state resulting from vitamin K deficiency to the mild, sometimes unrecognized, anorexia due to thiamine deficiency.

Vitamins may also be used as drugs not for deficiency states but to remedy an apparently unrelated disease or disorder. For example, the use of massive doses of vitamin D in the treatment of arthritis and large doses of vitamin C in allergy are in this category.

It is extremely difficult for one to separate truth and fiction in the welter of claims and statements concerning the use of vitamins. Even the physician, unless he is a dietary specialist, finds it difficult to keep himself properly informed in this field. It is no small wonder that the general public suffers from almost total confusion, particularly since vitamin products have become "big business" and the field

has been invaded by those who have exploited the gullibility of the American public for years.

Restricting vitamin sales to drug stores is a step in the right direction, since there is no other person who is in a better position to properly advise and guide the buying public in this field than the pharmacist. This does not mean that the physician is not the key person to whom all matters pertaining to health should be referred, but the physician is not and cannot be in a position to take care of the retail distribution of such products.

There is, however, a side to this restriction of vitamin sales which the pharmacist would do well to consider. With this economic advantage over other retailers there comes a very great responsibility, a responsibility so grave that it cannot be overemphasized. If the public receives no more careful and proper guidance in the purchase of vitamins from pharmacists than from any other retail outlet, then such legislation restricting vitamin sales to pharmacists will not long remain in effect.

The pharmacist should approach his responsibilities along this line with certain facts clearly in mind. It is his obligation to know exactly on what premise he can sell a vitamin product correctly and ethically "over the counter" to a customer. There are three such premises. Obviously if the customer has been directed by his physician to take a given product this is analogous to its having been prescribed, and the pharmacist should of course make such a sale without question. If the product is one of very high potency, repeated purchases should prompt the pharmacist to question the customer as to whether the physician is aware of his continued use of the product and, if not, strongly urge him to consult his physician before taking more. Then there is the customer who is the victim of advertising and who believes that vitamins will "cure" him of almost any ill that he possesses. This is the pharmacist's greatest challenge, because such a person will buy almost anything recommended and in most instances he may even, at first, resent being told the truth. Such a person *must* be told that the administration of vitamins is not a panacea and that no improvement can be expected unless his illness is due to a vitamin deficiency, which determination is clearly in the province of the physician. To sell vitamin A products for respiratory infections, and B_1 for loss of appetite, etc., without a physician's diagnosis is little short of criminal, since it may lead to the delay of proper therapy

until it is too late. At best the only product that can be sold conscientiously to such a person is a product that contains the minimum daily requirements of all the essential vitamins not as a cure-all but simply as a prophylactic measure. The customer must be told that the use of vitamins to *cure* disease or illness should only be attempted following a diagnosis and decision to do so by a reputable physician.

The third type of customer is one who feels quite well but takes some vitamin product because he thinks he should. In many instances, unless overdosage is practiced, this is simply an economic waste in that vitamins are used up without any gain on the part of the purchaser. Here, too, the pharmacist is obligated to direct the customer only to those products that are truly prophylactic with respect to all of the essential vitamins and not one of the all too common proprietaries containing a single vitamin or two vitamins in combination. If a product is taken as a prophylactic agent it must be truly such against all vitamin deficiencies since it is impossible for one to guess correctly which vitamin or vitamins are likely to be short in the diet. Frequently the laity takes, for example, a combination of A and D with the false assumption that full protection against a possible shortage of any and all vitamins is thereby assured.

The pharmacist therefore should be fully cognizant of his responsibilities in the sale of vitamins. He must be willing to lose many sales in order to discharge these responsibilities. His assistants must be cautioned against giving advice to customers on this subject which he himself cannot endorse. The challenge to pharmacy is great. If we meet it we shall raise ourselves in the eyes of both the medical profession and the public. If we do not, this legislation will be easily removed by those interests who strongly oppose it. Unless we give the public better protection than that given by non-pharmaceutical outlets such legislation cannot be justified nor will it long endure.

L. F. Tice.

PROGESTERONE, A HORMONE OF THE CORPUS LUTEUM

By Willard M. Allen, M. D.*

THE hormonal activity of the ovaries is dependent upon the production of at least two ovarian hormones. The first of these is estrogen, and the second, a progestogen. These two hormones produce the growth changes which take place in the reproductive organs and the breasts as the girl passes through puberty. The estrogenic hormone is active throughout the entire cycle whereas the second ovarian hormone, the progestogen, is active only in the second half of the cycle when the corpus luteum is functioning. The hormone of the corpus luteum is known chemically as progesterone and thus far only one chemically active progestogen has been obtained from the ovaries. Progesterone produces characteristic proliferative and secretory changes in the endometrium, these being produced under normal circumstances by the combined action of estrogen and progesterone. The changes which appear in the endometrium make possible the growth of the embryo in the uterine cavity and its attachment to the endometrium. Even after the embryo has become implanted, progesterone continues to be essential. In addition to the effect of this hormone on the endometrium, it also exerts specific effects on the uterine muscle and also characteristic effects on the breasts. Because of the role which progesterone plays in producing those conditions in the uterus which make implantation of the embryo possible, it has been called a progestational hormone.

The first experiments pointing toward the presence of a hormone in the corpus luteum were made in 1903 by Fraenkel (1). He observed that removal of the corpora lutea during the early stage of pregnancy in the rabbit invariably prevents implantation of the embryo. A little later, in 1907, Leo Loeb (2), working with the guinea pig, showed that the corpora lutea were essential for the production of the maternal portion of the placenta. By stimulating the uterus of

* Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, Missouri.

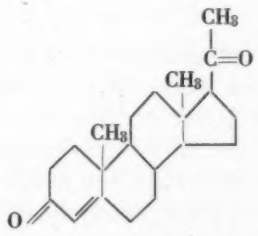
a guinea pig with active corpora lutea in the ovaries, he was able to produce deciduomata, a decidual tumor in the endometrium, very similar to that which occurs under normal circumstances when the embryo first burrows into the endometrium. Somewhat later (1908) it was observed by Hitschmann and Adler (3) that the endometrium of the woman undergoes cyclic changes and that during the second half of the cycle when the corpus luteum is present there are very characteristic effects which are never seen at any other time. These observations, of course, all pointed to the presence of a hormone produced by the corpus luteum.

The next step was to produce active extracts of the ovaries which might contain this hormone. Active extracts of the ovaries were, of course, made by various workers and the activity of such extracts was easily proved by showing that they induced growth of the uterus in immature animals or prevented atrophy following castration. This hormone, however, was subsequently found to be an estrogen, and it was likewise shown that it never produced those characteristic effects which the earlier observers had indicated must be due to a hormone of the corpus luteum. Finally when the crystalline estrogen, estrone, was obtained and found to be without any progestational activity, studies were started and carried out to isolate the missing hormone of the corpus luteum. This was accomplished in 1930 by Corner and Allen (4) when they discovered that active extracts of the corpus luteum produced specific progestational effects in the endometrium of castrated rabbits, and that when the experiments were carried out in the proper manner normal embryos could be obtained and pregnancy even maintained to term in rabbits castrated as early as eighteen hours after mating.

In 1934 the isolation of the pure compound was announced almost simultaneously by four different groups of workers, Butenandt (5); Slotta, Ruschig and Fels (6); Wintersteiner and Allen (7); and, Hartmann and Wettstein (8). The active principle was found to be a stereoid with the empirical formula $C_{21}H_{30}O_2$ and on further study and synthesis by degradation of pregnanediol and stigmasterol, Butenandt (9) was able to establish the structure.

Progesterone is a white crystalline substance, insoluble in water but soluble in vegetable oils, such as sesame, peanut and Mazola oils. The chemical characteristics and physical characteristics are as follows (10):

Preparation	M. P. (Kofler: uncorr.)	$(\alpha)^{25}D$ c = 0.257	$(\alpha)^{25}_{5,461}$	1% E(max.) 1 cm,	λ max.
Second International Standard	130°-132°	+191°	+226°	530	2,410
First International Standard	128.5°-129°	+192°	+232°	510	2,430



$C_{21}H_{30}O_2$

The international unit of progesterone is defined as the biological activity of one milligram of the international standard. At the present time the best method for determining the activity of progestational substances is that of Corner and Allen (4). This method consists of the injection of the material into sexually mature rabbits, castrated the day after mating. The injections are made daily for a period of five days and the animal is sacrificed on the sixth day. The uterus is then removed and microscopic sections taken of the uterus. The degree of activity is determined by observing the progestational effects which are produced in the endometrium. Unknown preparations should be compared with the international standard for accurate bioassay.

The clinical uses of progesterone should, of course, be reserved for those conditions in which a deficiency of the corpus luteum hormone is known to be present. It seems probable that the most extensive clinical use of the material at the present time is for the prevention and treatment of threatened miscarriage. This use is, of course, based upon the observation that removal of the ovaries or of the corpora lutea in many laboratory animals is followed by abortion, and also upon the observation that the administration of progesterone to such

castrated animals, especially rabbits, will in practically every instance prevent abortion (11). The clinical use, based upon this laboratory finding, is, however, on less secure foundation. Many spontaneous abortions undoubtedly occur as a result of progesterone deficiency. However, in many such instances the embryo is primarily at fault and this is only Nature's way of causing the uterus to expel an abnormal fetus. The administration of progesterone does not long prevent the expulsion of such a fetus so that ordinarily the use of this hormone does no harm even though in many instances it undoubtedly does no good.

The second use for progesterone is found in the treatment of functional uterine bleeding, especially that which occurs in young women with hyperplasia of the endometrium (12). In hyperplasia the endometrium shows the effects of continued action of estrogen without the effects of progesterone, and it is known that the ovaries do not contain any corpora lutea. This, therefore, is one condition in which there is a proven deficiency of progesterone. The intramuscular administration of 5 to 10 mgm. per day of progesterone for a week or ten days produces some secretory changes in the endometrium and oftentimes the bleeding disappears during therapy. About two days after the administration of the hormone is discontinued there is exacerbation of bleeding and the endometrium breaks down in much the same manner as it does during a normal menstrual period. This plan of therapy results in extensive shedding of the hyperplastic endometrium. Following a single course of therapy the bleeding may recur but in many instances normal cycles are present for many months, sometimes indefinitely.

Still another use for progesterone is in the treatment of secondary amenorrhea (13). Amenorrhea of this sort is due to failure of ovulation and hence to progesterone deficiency. The administration of progesterone in this condition for a matter of seven to ten days, or in some cases even only three or four days, is usually followed by a menstrual period. Just as in functional uterine bleeding many of these patients have fairly normal cycles after a single course of treatment with progesterone.

One other use for progesterone is found in the treatment of essential dysmenorrhea. One of the characteristic effects of progesterone is to produce relaxation of the uterine muscle of certain laboratory animals (14). It does not seem to produce such relaxation, however,

in the human uterus. Its effect on laboratory animals, however, has led to its use in the treatment of dysmenorrhea and in many instances has been found beneficial although certainly not curative.

Progesterone is inactive when taken orally and hence has to be given by intramuscular injection. It is fairly rapidly metabolized and consequently should be given at least once per day if effective results are to be obtained. More recent observations indicate that it is fairly well absorbed from the mucous membrane of the mouth. Preparations in propylene glycol may be taken as drops beneath the tongue and slowly absorbed tablets can be used in the form of linguets.

There is one other compound closely related to progesterone which is active by mouth. This is anhydro-hydroxy-progesterone (ethinyl testosterone) (15). The amount of this compound necessary to produce effects equivalent to progesterone is not entirely clear at the present time. It seems probable, however, that it takes about ten times as much of this orally active preparation to produce a characteristic effect as it does of progesterone when given intramuscularly. So far as is known this compound has all of the physiological characteristics of progesterone and can be used whenever it is more convenient to have the patient take oral medication rather than to receive intramuscular injections.

BIBLIOGRAPHY

1. Fraenkel, L.: *Arch. f. Gynäk., 68*:433 (1903).
2. Loeb, Leo: *Zentralbl. f. Allg. Path., 18*:563 (1907).
3. Hitschmann, F., and Adler, L.: *Monatschr. Geburtsh. u. Gynäk., 27*:1 (1908).
4. Corner, G. W., and Allen, W. M.: *Am. Jr. Physiol., 88*:326 (1929).
5. Butenandt, A.: *Ber. chem. Ges., 67*:2085 (1934).
6. Slotta, K. H., Ruschig, H., and Fels, E.: *Ber. chem. Ges., 67*:1947 (1934).
7. Wintersteiner, O., and Allen, W. M.: *Jr. Biol. Chem., 107*:321 (1934).
8. Hartmann, M., and Wettstein, A.: *Helv. chim. Acta, 17*:878 (1934).
9. Butenandt, A., and Schmidt, J.: *Ber. chem. Ges., 67*:1901 (1934).
10. *Bull. Health Organization of the League of Nations, 10*:86 (1942).
11. Allen, W. M., and Corner, G. W.: *Am. Jr. Physiol., 88*:340 (1929).
12. Allen, W. M., and Heckel, G. P.: *Am. Jr. Ob. and Gyn., 44*:984 (1942).
13. Zondek, B., and Rozin, S.: *Am. Jr. Ob. and Gyn., 40*:391 (1940).
14. Allen, W. M., and Reynolds, S. R. M.: *Am. Jr. Ob. and Gyn., 30*:309 (1935).
15. Inhoffen, H. H., and Hohlweg, W.: *Naturwisschaften, 26*:96 (1938).

ESTIMATION OF TOTAL H_2S METALS IN IRON SALTS

By Harold Rosenblum and Joseph Rosin *

A PROCEDURE similar to the general U. S. P. test for heavy metals or to the test for H_2S metal impurities in ferrous sulfate has not been available for ferric salts because of the formation of sulfur in the reaction between a ferric salt and hydrogen sulfide. Treatment of the ferric salt solution with an excess of ammonia as given in the U. S. P. for Reagent Ferric Chloride, or in the N. F. VII for Solution of Ferric Chloride, yields only copper and silver if present. Lead, although much less than formerly, is almost an invariable impurity in iron salts, and in the extraction with ammonia water the lead is adsorbed on the ferric hydroxide. Copper and lead are the foreign metals most commonly present in iron salts, but the possibility of the presence of small amounts of heavy metals, other than lead, which are insoluble in ammonia and retained by the ferric hydroxide, is not precluded.

Small quantities of lead can be determined with a very satisfactory degree of accuracy by the dithizone method, and copper, by extraction with ammonia, but since other H_2S metals than copper or lead may be present, the total of such metal impurities will not be indicated. Moreover, the two separate determinations, i. e., of the lead and the copper, consume considerable time. For the purpose of insuring a proper degree of purity of ferric salts a convenient single test which would include all the H_2S metal impurities is, therefore, very desirable.

It is evident that if the ferric salt can be reduced to ferrous with an agent that will not interfere with the subsequent treatment of the solution with H_2S the U. S. P. method, suitably modified, could be applied with a reasonable degree of accuracy to the determination of the total of these metals. We found in hydroxylamine hydrochloride an agent eminently suitable for this purpose. Hydroxylamine sulfate can probably be used equally as well. Hydroxylamine hydrochloride rapidly reduces ferric salts to ferrous on heating approximately as follows: $4FeCl_3+2NH_2OH.HCl=4FeCl_2+N_2O+H_2O+6HCl$.

A moderate excess of hydroxylamine is beneficial in that it aids in maintaining the iron in the ferrous state. The solution thus reduced

*From the Laboratories of Merck & Co., Inc., Rahway, N. J.

and after neutralizing with ammonia as indicated later on, can then be treated wtih hydrogen sulfide and the intensity of the dark color matched against controls made with standard solutions of copper or lead.

In the reaction between a ferric salt and hydroxylamine hydrochloride free acid is produced both from the ferric salt and from the hydrogen chloride of the reagent. Free HCl, more so than free sulfuric acid, is known to interfere with the colorimetric determination of lead by hydrogen sulfide and it is, therefore, necessary to neutralize the free acid. The preferred neutralizing agent is ammonia water. This neutralization, however, using litmus paper as an indicator is difficult because of the precipitate that forms after the dropwise addition of ammonia water. We have found it more convenient to add an amount of ammonia water slightly less than that calculated from the quantity of ferric salt taken for the test. Using 0.5 Gm. of the ferric salt for the test, the following volumes of 10 per cent ammonia water are to be used:

Ferric chloride, $FeCl_3.6H_2O$	0.50 cc.
Ferric sulfate, $Fe_2(SO_4)_3$—about 20% H_2O	0.55 cc.
Ferric ammonium sulfate, $FeNH_4(SO_4)_2.12H_2O$	0.25 cc.

Since copper is the predominant foreign heavy metal in iron salts, it follows that a copper standard should be used and the total H_2S heavy metals expressed as copper. Copper is also preferable as a standard for comparison for the reason that the color it produces with H_2S is not as intense as that produced by an equal quantity of lead. The ratio between copper to lead sulfide with respect to intensity of color is approximately 10:7, which means that about 10 parts of copper are required to give the same color intensity as 7 parts of lead.

Procedure—The following procedure described for ferric chloride applies to other water-soluble inorganic ferric salts, differing only in the volume of ammonia water to be used as indicated before. Water-insoluble compounds, like ferric oxide, may be made soluble by evaporation with hydrochloric acid.

To a solution of 0.5 Gm. of ferric chloride in 30-35 cc. of water contained in a small Erlenmeyer flask or to a corresponding aliquot of a stock solution diluted to 35 cc. with water, 0.4 Gm. of reagent hydroxylamine hydrochloride is added and agitated

until dissolved. This is followed, while swirling the solution, by the dropwise addition of 0.50 cc. of 10 per cent ammonia water. After all the ammonia has been added any iron hydroxide that may have been formed on the sides of the flask is rinsed down with a few cc. of water, the solution heated on the steam bath until practically colorless, then gently boiled for about a minute. The solution is promptly cooled, diluted to 40 cc. with water, 10 cc. of hydrogen sulfide T. S. added, and the color matched in Nessler tubes against controls prepared with a standard cupric sulfate solution containing 0.5 cc. of N/10 HCl and to which the same quantity of hydroxylamine hydrochloride has been added as in the test.

The amount of heavy metals possibly introduced by the ammonia water is negligible.

A correction should be made for the color derived from the ferrous salt. This correction need not be made each time, but it can be established once and for all by treating the same quantity of the ferric salt, e. g. chloride or sulfate, etc., as in the test with hydroxylamine hydrochloride, diluting to 50 cc. with water and comparing the color with that of a standard cupric sulfate solution diluted to 40 cc. and 10 cc. of hydrogen sulfide T. S. added. Inasmuch as the colors of the two solutions differ in shade, they should be rather compared for intensity. In our experience the color attributable to the ferrous iron, under the conditions of the test described before, amounts to from 5 to 10 parts per million.

The foregoing method is not directly applicable to organic iron salts, but it can be made available for these by destroying the organic matter as illustrated by the procedure described below for ferric ammonium citrate. This method is also not available for complex ferric compounds, such as the citrophosphates, because the ferric iron in these salts is not reducible by hydroxylamine and the interference of the phosphate ions.

Ferric ammonium citrate—One-half Gm. of the salt is placed in a 125 cc. Erlenmeyer flask, 1-2 cc. of sulfuric acid is added, and the mixture gently heated over a wire gauze until it turns black. Four cc. of nitric acid is added a few drops at a time, heating the mixture for one-half to one minute after each addition. After all the nitric acid has been added, it is heated to strong fumes of SO_3, then cooled, 2 cc. of water cautiously added and evaporated

almost to dryness. After cooling, 50 cc. of water and 0.5 Gm. of reagent hydroxylamine hydrochloride are added and heated until the solution is practically colorless. It is then cooled and 10 per cent ammonia water added dropwise, until a slight turbidity is produced, and the mixture boiled gently until the solution is clear and practically colorless. It is immediately diluted with water to 80 cc., and to 40 cc. of the dilution 10 cc. of hydrogen sulfide T. S. are added. The color is matched with controls made with standard cupric sulfate solution treated in the same manner as the sample. In neutralizing the control solution phenolphthalein indicator may be used, adding sufficient ammonia to produce a pink color, which is then discharged by the addition of 2 cc. of tenth-normal hydrochloric acid. Correction should also be made for the amount of color due to the ferrous iron.

The heavy metal content of several ferric salts as determined by the hydroxylamine method is illustrated in the following tabulation:

Ferric Salt Used	p. p. m. Pb found by dithizone	p. p. m. total H_2S metals as Pb by hydroxylamine method	p. p. m. Pb added	p. p. m. total found as Pb by hydroxylamine method	p. p. m. total H_2S metals as Cu by hydroxylamine method	p. p. m. Cu added	p. p. m. total found as Cu by hydroxylamine method
Reagent ferric chloride No. 1	5	25	50	75	30	50	80
Reagent ferric chloride No. 2	7	..	..	..	40	50	85
Reagent ferric sulfate	5	50	50	100	60	50	110
Reagent ferric ammonium sulfate	3	30	50	80	40	50	90
Ferric ammonium citrate, brown	10	150	300	450	..	..	..
Ferric ammonium citrate, green	30	200	300	500	..	..	..

In testing ferrous sulfate for copper and lead the U. S. P. XII directs to dissolve 0.5 Gm. of the substance in 40 cc. of recently boiled and cooled distilled water, adding 1 cc. of diluted sulfuric acid and after diluting with water to 50 cc. one-half of the dilution is treated with 10 cc. of H_2S and the other half with 10 cc. of water alone. It is required that the portion treated with H_2S should not be darker than the portion without H_2S. Under Exsiccated Ferrous Sulfate the U. S. P. requires that the material should comply with the tests

for ferrous sulfate, allowance being made for the difference in water content.

Exsiccated Ferrous Sulfate, because of oxidation during the drying, invariably contains ferric salt; consequently when its solution is treated with H_2S sulfur is precipitated, obscuring any color derived from copper or lead and rendering the test useless. Treatment of the solution, previous to addition of H_2S, with hydroxylamine reduces the ferric to ferrous, thus removing the interference from ferric salt, and makes available a quantitative determination of the H_2S heavy metals. The procedure is as follows:

To 0.5 Gm. of the Exsiccated Ferrous Sulfate, 10-15 cc. of water and 4 cc. of tenth-normal hydrochloric acid are added and warmed for a few minutes on the steam bath to hasten solution (hydrochloric acid is preferable to sulfuric acid because it dissolves the basic salt more readily than the latter acid). The solution is diluted with water to 80 cc., filtered, 50 mg. of hydroxylamine hydrochloride are added to the filtrate and boiled for about one minute. After cooling under the tap, sufficient water is added to restore the volume to 80 cc. To 40 cc. of the dilution, 10 cc. of hydrogen sulfide are added, and after one minute the color produced is compared with controls prepared with known amounts of copper or lead and containing 2 cc. of tenth-normal HCl.

A sample of exsiccated ferrous sulfate tested for copper or lead according to the U. S. P. method was estimated, with great uncertainty, to show 10 to 20 p. p. m. as lead, but when the test was made as described, the total heavy metal content as lead was found to be 120 p. p. m., and as copper 200 p. p. m.

The U. S. P. test for copper and lead in ferrous sulfate, by allowing no darkening of the solution by hydrogen sulfide T. S. limits, in our estimation, these metals to about 30 p. p. m. Ferrous sulfate U. S. P. of commerce generally does show a darkening in the test. Inasmuch as the foreign heavy metal is largely copper, and since copper in small amounts is relatively innocuous, it is recommended to the U. S. P. Revision Committee to liberalize the limit for the foreign heavy metals content. It is also recommended that the test be made quantitative, using the procedure recommended for Exsiccated Ferrous Sulfate, except that 2 cc. of tenth-normal hydrochloric acid can be used instead of 4 cc.

A PRELIMINARY REPORT ON A SIMPLIFIED TECHNIC OF THE RUGE VIRULENCE TEST WITH SOME CLINICAL OBSERVATIONS

By Louis Gershenfeld, D. Sc.,* and Morton Reese Cohen, M. D.**

IN a study of the deaths following 6022 gynecologic operations, Greenhill (1), makes a plea for more preoperative preparation and laboratory tests. He also suggests the routine employment of the sedimentation test in inflammatory cases. While there is a wealth of favorable literature concerning the sedimentation test, this procedure has been adopted and later abandoned in many clinics.

Gellhorn (2), in a contribution to the treatment of septic abortion states, "At the present stage of our knowledge we are unable to foretell the virulence of an infection." This writer is either unfamiliar with or has underestimated the value of a procedure which has enriched the European literature: the Ruge-Philipp virulence test. Very little work concerning this practical test has appeared in the English written literature. Simunich (3), in a comparison of this test with the sedimentation time test concludes that:

1. "The sedimentation test is not a reliable guide in the determination of the time for safe operation of adnexal disease" and that,

2. "The Ruge virulence test is of value in the prognostication of post-operative morbidity and mortality if the operation takes place at the site of the organisms, usually the cervix." He also states that the Ruge virulence test is of undoubted value in the determination of the time for safe operation of the combined cervico-abdominal type.

Kolmer (4), writing of the diagnosis and treatment of surgical septicemia, stresses the value of the estimation of the bactericidal and bacteristatic activity of the blood.

The test as introduced by Carl Ruge (5), was a direct microscopic observation. He mixed the vaginal secretion with fresh defibrinated blood of the patient. The mixture was studied on a sterile slide ringed with wax. In this method a warm stage was employed

* Department of Bacteriology of the Philadelphia College of Pharmacy and Science.

** Department of Gynecology, Mount Sinai Hospital.

and one could see either the gradual arresting and disintegration of the bacteria or a growth in number of the organisms. The material was studied for 4 to 8 hours and the organisms were designated virulent or avirulent depending upon the above-mentioned findings.

Ernst Philipp (6), modified the test and employed it to determine the virulence of organisms in bacteremia of puerperal sepsis. He took the blood from the patient during a chill and poured 1.5 cc. on an agar plate while the remainder was placed in the incubator. At stated intervals of 1, 2, 3, 6 and 12 hours he poured the same quantity of blood taken from the incubator upon agar plates. He therefore had plates which were made from blood which had been poured immediately after withdrawal from the patient and after 1, 2, 3, 6 and 12 hours incubation. These were examined and the colonies counted. A steady increase in the number of colonies in direct ratio to the chronological order of the plates spoke for a virulent organism and a stormy convalescence. If a decrease in the number of colonies with a final sterile plate was observed, it meant an avirulent organism and a favorable prognosis.

The disadvantages of these methods are obvious. In the original Ruge method a warm stage was required with careful observation of a fixed field. Hadijakis (7) employed this method but used the incubator for his slides instead of the warm stage. Loss of the field meant a repetition of the test with resultant discomfort to the patient and waste of valuable time.

In the Philipp procedure there is the economical factor of the employment of 12 plates if controls are to be used. The 12 hours consumed until the last plate is poured, in addition to at least 48 hours for observation of growth, almost prohibits this method as a prophylactic measure. If virulent organisms are found there is the additional period of several days required for the preoperative preparation of the patient.

Frohlic (8) combined both of the methods in that he employed a vaginal secretion-bouillon-blood mixture and poured plates from this immediately and after 3 and 6 hours. He lessened the number of plates but still required 48 to 72 hours for observation. He greatly enhanced the value of the procedure by using both aerobic and anaerobic methods.

Doderlein (9) first focused attention upon the biologic and pathologic aspects of the vaginal flora. This subject was further

studied and made popular by Schroeder. Many European clinics take routine smears of their gynecologic patients who are then classified as to the degree of "Reinheitkeit" of the genital tract.

During the performance of the Ruge virulence test we also made smears which were stained by Gram's method. These are not required for the test but in addition to Brain veal infusion cultures, were reserved for possible aid should we become interested in any morphologic aspect while counting the bacteria in the blood spreads.

The purpose of this contribution is to present a detailed practical technic of applying this test; a record of observations in a series of cases in which the test as set forth was performed is also included. It is hoped that this method will find favor because of its simplicity and the small time factor required in the performance of the test.

Technic

Materials Required:

Sterile Graves speculum; sterile cotton applicators in container; sterile 10 cc. syringe with needle; sterile 60 cc. Erlenmeyer flask containing glass beads; sterile 1 cc. pipette graduated in 0.1 cc.; two sterile test tubes; special platinum loop of 0.01 cc. capacity; spreader; two slides ruled as shown; and staining jar containing the dilute carbol-fuchsin solution.

Procedure

The genital region is shaved, washed with tincture of green soap and rinsed with sterile water. The labia are widely separated and the sterile speculum carefully inserted. The cervix is brought into view and the sterile cotton-tipped applicator gently inserted into the cervical canal. It is withdrawn and carefully returned to the sterile test tube container.

Under aseptic conditions, as for blood culture, 10 cc. of blood are withdrawn from the cubital vein and carefully placed into the flask containing the glass beads. The flask is shaken for at least ten minutes to prevent clotting of the blood. Inasmuch as the above is performed at the bedside of the patient, great caution is necessary to prevent contamination.

In the laboratory, 1 cc. of the blood is removed from the flask by means of the sterile pipette and 0.5 cc. of it is allowed to run into a sterile test tube. The other 0.5 cc. is deposited in another sterile test tube. One of these tubes containing blood is inoculated by thor-

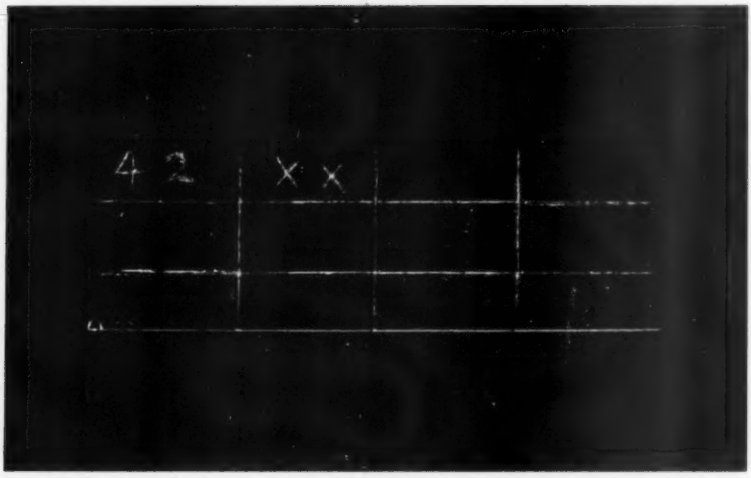


FIGURE 1. Micro slide ruled and numbered by carborundum pencil. The four middle spaces are used for the immediate, first, second, and after the third hour's incubation. The extra spaces above and below may be used in case a central spread is unsatisfactory. The xx following the number designates a control slide, while a single x is used for the innoculated slide.

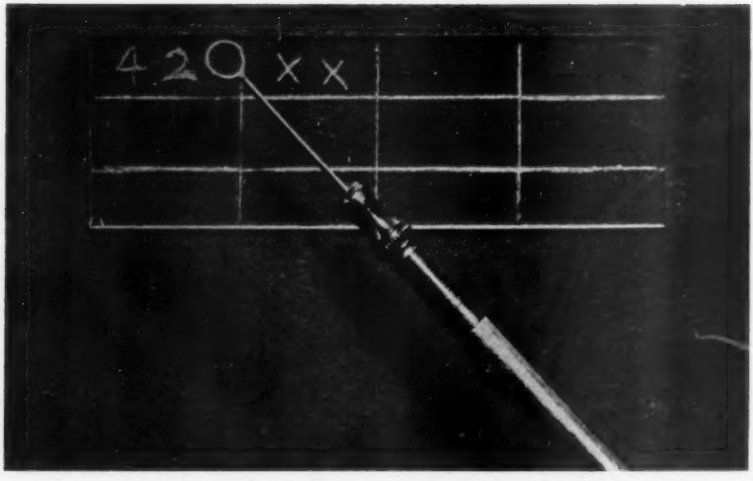


FIGURE 2. Heavy gauge platinum loop of 0.01 cc. fixed capacity as used in Brew and Breed method of counting bacteria in milk. May be obtained from Arthur H. Thomas Co., Philadelphia.

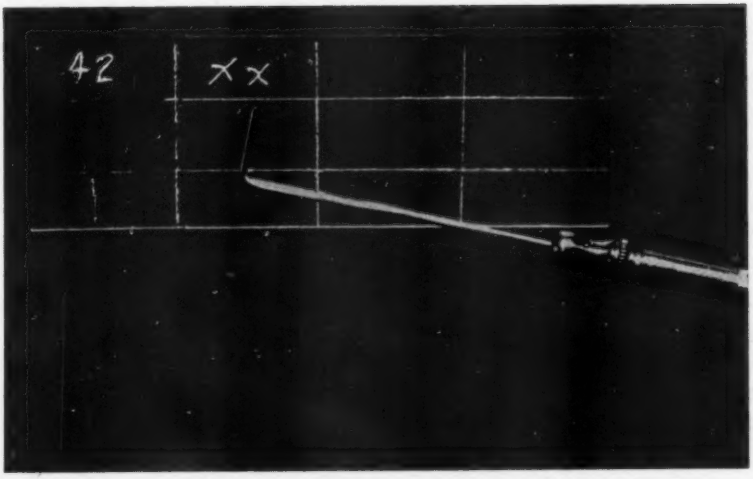


FIGURE 3. Ruled slide showing spread made from blood drop by spreader made from a bent paper clip and inserted into needle holder.

oughly washing off the cervical secretion from the applicator in the blood. The second test tube with blood is retained as a control.

The inoculated tube is thoroughly shaken and the first spread is made by means of the special loop and spreader. On another slide a similar spread is made of the control blood. The tubes are then incubated at 37.5° C. and spreads are made from both tubes at the end of the first, second and third hours. The slides are well dried without fixing in the flame (since rapid heat has a tendency to cause cracks to appear in the spread). The slides are then placed in the staining jar containing diluted carbol-fuchsin and allowed to remain for three minutes, after which they are gently rinsed in water and allowed to dry. Under the oil immersion objective, the bacteria are counted as mentioned later, employing preferably a mechanical counter. An increase or decrease of the bacterial content is noted in the hourly spreads of blood.

In the evolution of this procedure many problems arose. Early in the investigation, we attempted to take advantage of the work recorded by Adair et al. (10), in which they recommended the employment of the apparatus described by Harris and Brown (11). The tubes were made up and worked very well. However, since this study is one of virulence and immunity rather than a determinative

problem, the special tubes were abandoned. Since the genital tract may be invaded by the same accidental contaminants preoperatively, it was decided to consider reasonable care to prevent contamination as being sufficient for our purpose.

The form of the slide ruling was adopted after much experimentation with the area of the spread. It was shown that the chosen area was best suited for a spread of blood made from a loop of the given capacity.

The stain was adopted after study. It is made by diluting one part of carbol-fuchsin with nineteen parts of distilled water. Three minutes' staining is usually sufficient, but the spread may be held to the light and if understained the natural color of the blood will be seen; it may then be replaced in the stain for another minute if necessary.

In counting the bacteria, twenty fields are examined. The organisms stand out very clearly against the background of blood. There is generally no doubt as to the increase or decrease of the bacterial content although there may be an occasional questionable finding.

The modification of the Ruge virulence test described above was carried out in a series of thirty cases. While this study was undertaken primarily to develop a practical and simple procedure, the inclusion of the clinical work and findings may be of interest. The operative cases consisted of the following types:

A. *Abdominal operations*

Supra-vaginal hysterectomies
Salpingo-oophorectomies
Salpingostomy
Myomectomy
Ventral and other suspensions of uterus

B. *Vaginal operations*

Vaginal cyst
Perineorrphaphies
Amputations of cervix
Trachelorrhaphies
Dilatation and curettage (diagnostic)
Implantation of radium
Therapeutic interruption of pregnancy (tuberculosis)

Case Histories of the Patients with Positive Tests

F. T. Case No. 80583, Slide No. 3

This patient, aged 44, was admitted October 24, 1933, complaining of pain in the lower abdomen of 6 months' duration. On admission her temperature was normal. Six days after admission she was operated upon under general anesthesia, a bilateral salpingo-oophorectomy, ventral suspension and appendectomy being performed. The day following operation her temperature rose to 101.4° F. by axilla. Her temperature on the fourth day after operation was 103.4° F. by mouth. Patient expired on the sixth day after operation, her temperature having reached 107° F. that day. *Pathological report:* Chronic salpingitis, plastic exudate at site of suspension and partial intestinal obstruction.

A. P. Case No. 82323, Slide No. 14

This patient, aged 56, was admitted January 25, 1934, complaining of vaginal bleeding, abdominal discomfort and loss of weight. Her temperature on admission was 99° F. by mouth. Four days after admission she was operated upon, a diagnostic curettage being performed. A diagnosis of adeno-carcinoma having been made, a supra-vaginal hysterectomy and bilateral salpingo-oophorectomy were performed on February 15, 1934. An appendectomy was also done at the same time.

Her convalescence was complicated by a rise in temperature to 101° F. on several occasions. She remained in the house for 27 days and was discharged with a normal temperature. *Pathological report:* Primary adeno-carcinoma of the uterus.

L. G. Case No. 82340, Slide No. 13

This patient, aged 32, was admitted on January 26, 1934, complaining of long standing lower abdominal pain. Her temperature on admission was 99.2° F. She was operated upon three days after admission when a trachelorrhaphy, right salpingo-oophorectomy, plastic on left tube, ventral suspension, appendectomy and separation of adhesions were performed. The temperature started to rise 24 hours after operation and soon became septic in type ranging from 96.2° F. to 102° F. Her convalescence was rather stormy, necessitating a blood transfusion on the fifteenth day after operation. The temperature slowly fell to 100° F. at which time the patient left the hospital against advice on the twenty-eighth day after operation. *Pathological*

report: Ovary; corpus luteum cyst, intraparenchymal hemorrhage with oedema. Tube:—Subacute salpingitis; chronic appendicitis.

C. D. Case No. 83625, Slide No. 37

This patient, aged 44, was admitted to the hospital April 8, 1934, with complaint of vaginal bleeding. Her temperature on admission was 98° F. On April 23, 1934, a bilateral salpingo-oophorectomy and supra vaginal hysterectomy were performed. Her temperature gradually rose to 103° F. on April 28. It fluctuated daily and slowly fell to 100° F. on May 7. *Pathological report:* Papillary cystadenoma of the ovary with carcinomatous changes.

R. K. Case No. 83065, Slide No. 10 *

This patient, aged 44, was admitted on March 6, 1934, complaining of vaginal bleeding. Her temperature on admission was 98.8° F. She was operated upon the next day when a D. & E. was performed together with an amputation of the cervix. Her convalescence was uneventful; the highest temperature reached was 100° F. three days after operation. She was discharged four days after operation with a temperature of 97.6° F. *Pathological report:* Lacerations and chronic cervicitis; chorionic villi and decidual tissue.

Case Histories of Patients with Negative Tests, but with Morbidity

J. B. Case No. 82115, Slide No. 1

This patient, aged 36, was admitted to the hospital on January 13, 1934, complaining of excessive vaginal discharge. The temperature on admission was 98.2° F. The temperature on the fourth day after operation was 101.2° F. This patient had an amputation of the cervix, excision of a vaginal cyst and a perineorrhaphy on January 16, 1934. Her convalescence was complicated by the rise in temperature as noted above. She was discharged with a temperature of 98° F. on February 1, 1934. *Pathological report:* Chronic cervicitis and inclusion cyst of vagina.

R. W. Case No. 80362, Slide No. 4

This patient, aged 53, was admitted to the hospital on October 25, 1933, complaining of abdominal pain. Her temperature on ad-

* This is a case where the clinical course did not coincide with the positive result of the Ruge test.

mission was normal. A hysterectomy, bilateral salpingo-oophorectomy and perineorrhaphy were performed. On the second day after operation her temperature was 101.8° F.; this rose to 102° F. on the twenty-first day after operation. She was discharged on November 23, 1933, with a normal temperature. *Pathological report:* Chronic salpingitis.

Summary

Positive results were obtained in five of the twenty-five cases examined. Of these, four coincided with the clinical picture as evidenced by a rise of temperature and other manifestations of morbidity. Two cases with negative results showed some morbidity.

The Ruge virulence test is presented and details of a modified technic are given. This test is advocated for more popular adoption as a means in the prevention of postoperative morbidity and mortality. There is no question as to the value of the test. The results herein recorded are in accordance with those obtained by other workers. From a practical standpoint, considering especially the economic viewpoint, it is obvious that a few days spent in attempts to attenuate the virulence of the organisms present are well utilized; an additional day or two spent in preoperative care will often prevent a week or two of post-operative morbidity.

REFERENCES

(1) Greenhill, J. P.: A Study of the Deaths Following 6022 Gynecologic Operations, *Am. Jour Obst. & Gynec., 16*:4 (1928).

(2) Gellhorn, George: The Treatment of Septic Abortion, *Am. Jour. Obst. & Gynec., 24*:183 (1932).

(3) Simunich, Wm. A.: A Comparison of the Sedimentation and Ruge Virulence Tests in 150 Gynecologic Cases, *Am. Jour. Obst. & Gynec., 23*:5 (1932).

(4) Kolmer, John A.: Laboratory Aids in the Diagnosis and Treatment of Surgical Septicemia, *J. Lab. & Clin. Med., 16*:685 (1931).

(5) Ruge, Carl, II: Virulenzbestimmung der Streptokokken, *Med. Klin., 200* (1923).

(6) Philipp, Ernst: Virulenzbestimmung von Blutkeimen, *Münch. Med. Wschr., 493* (1923).

(7) Hadijakis, George E.: Die Bedeutung der Virulenzprüfung der Streptokokken für die Geburtshilfe und Gynakologie, *Wiener Med. Wschr., 42*:1241 (1926).

(8) Frohlich, Walter: Über den postoperativen Verlauf nach gynakologischen Operationen mit vorheriger bakteriologischer Sicherung gegen endogene Infektionen, *Arch. f. Gynak., 148*:748 (1932).

(9) Doderlein: Das Scheidensekret (Leipzig, 1892).

(10) Adair et al.: A Bacteriologic Study of Technics for Taking Vaginal and Cervical Cultures, *Am. Jour. Obst. & Gynec., 25*:551 (1933).

(11) Harris, John W., and Brown, J. Howard: A Method of Obtaining Vaginal Secretion for Bacteriologic Examination Without the Possibility of Vulval Contamination, *Am. Jour. Obst. & Gynec., 11*:497 (1926).

SELECTED ABSTRACTS

Sulfathiazole, Effect of Procaine on Bacteriostatic Activity of. D. Casten, J. J. Fried and F. A. Hallman. *Surg. Gynaec. Obstet. 76,* 726 (1943); through *Quart. J. Pharm. & Pharmacol. 17,* 69 (1944). Experimental wounds on rabbits were inoculated with *Staphylococcus aureus;* all of the control wounds became heavily infected, and thirteen out of thirty-three similar wounds treated with a single local application of sulfathiazole became mildly infected.

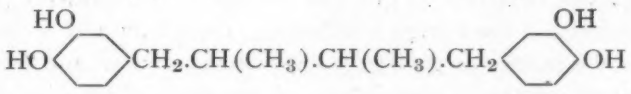
However, when the muscle around such an inoculated wound was infiltrated with 1 mil of a 2 per cent solution of procaine hydrochloride prior to treatment with sulfathiazole, in every case the wound became infected. The antagonistic action of procaine toward the bacteriostatic activity of sulfathiazole is due to the fact that the former is a derivative of *p*-aminobenzoic acid, and its use is therefore contraindicated in wounds to which sulfathiazole is applied locally.

Penicillin, Synergistic Effect of p-Aminobenzoic Acid and Sulphapyridine on. J. Ungar. *Nature, Lond., 152,* 245 (1943); through *Quart. J. Pharm. & Pharmacol. 17,* 54 (1944). The addition of small quantities of *p*-aminobenzoic acid to sodium penicillin increased the inhibition of growth of *Bacillus subtilis;* this effect, however, was less marked on *Staphylococcus aureus,* and absent on *Streptococcus haemolyticus.* When sulfapyridine, in quantities sufficiently small to be without effect themselves on the organism, was added to sodium penicillin, the increased inhibition of growth was more marked than that noted in the case of *p*-aminobenzoic acid.

Mice infected with *S. haemolyticus* or *S. aureus* exhibited a greater survival when penicillin and sulfapyridine were injected simultaneously than when only one of the substances was administered. This effect is believed to be due to a synergistic action on the organism, rather than to a chemical reaction between the two substances.

Antioxidant Properties of Nordihydroguaiaretic Acid. W. O. Lundberg, H. O. Halvorson and G. O. Burr. *Oil and Soap 21,* 33 (1944); through *Analyst 69,* 223 (1944). Nordihydroguaiaretic acid was obtained in a yield of about 7 per cent (calculated on the dry weight) from *Larrea divaricata,* a common desert plant. Its antioxidant properties were found to compare favorably with those of other effective inhibitors of the phenolic type. At concentrations up to about 0.1 per cent the acid had no deleterious effect on the quality of lard. It was noted that its stabilizing action on fats was, to some extent, still present in baked products, and that its efficacy is enhanced by the presence of vitamin C.

Nordihydroguaiaretic acid has the following structural formula:



It is very slightly soluble in water, but is appreciably soluble in hot fats, from which it does not crystallize on cooling. Its solubility in fats is greater than that of hydroquinone, but less than that of the tocopherols.

The Hydrolysis of Diamorphine Hydrochloride. G. A. Storey. *Pharm. J. 99,* 44 (1944). The observation that a solution of diamorphine hydrochloride prepared for injection deteriorated on standing for several weeks, liberating morphine in excess of the 1.5 per cent permitted by the B. P., led to an investigation of the effect of storage and sterilization on the rate of hydrolysis of the compound in solution.

After one month of storage the amount of morphine present was 5.1 per cent; after seven months it was 19.0 per cent. The amount of morphine resulting from the hydrolysis of diamorphine hydrochloride solution after sterilization by heat was found to be as follows: steaming for 30 minutes, 3 per cent; autoclaving for 20 minutes at 15 lbs., 5.7 per cent; heating at 160° C. for 90 minutes, 52.5 per cent.

The pH of the solutions was decreased both by sterilization and storage. The freshly prepared solution had a pH of 5.2; after steaming, it was 4.4; and after autoclaving, 4.1. After standing for three months the solution had a pH of 4.6; after seven months, 4.1.

The Stability of Vitamin C in Blackcurrant Syrup. F. Wokes and J. D. Organ. *Pharm. J. 99,* 44 (1944). The effect of storage temperature and of exposure to air and light on the vitamin C content of a fresh lot of blackcurrant syrup was studied under varied conditions. The syrup was stored in four-ounce bottles of either clear or amber glass; some of these were completely filled, while the remainder were only partially filled, containing either air or nitrogen above the syrup. Storage was under a range of temperature from 0° to 40° C. Both types of bottles were exposed to direct sunlight and to diffused light, and in addition the effect of a light-proof paper wrapper for the bottles was studied.

At intervals determinations were made for total vitamin C and for apparent vitamin C. The rate of loss of the former increased with elevation of the storage temperature, though the rate of development of the latter was greater at higher temperatures, and when the preparations contained much pectin. Compounds of the reductic acid type may be part of the apparent vitamin C.

Syrup stored in clear glass bottles exposed to sunlight showed a rate of loss two and a half times as great as that observed for samples stored in identical bottles wrapped in light-proof paper. Amber glass did not entirely prevent the loss.

The rate of loss in bottles not more than half-full was three times as great as that observed in those which were completely filled. The replacement of air by nitrogen reduced the loss. Completely filled bottles stored for ten months in the refrigerator showed a loss of 7 per cent.

It is recommended that all fruit syrups used as a source of vitamin C should be stored in a cool, dark place.

SOLID EXTRACTS

Paragraphs of Scientifically Interesting Facts

A new member of the sulfonamide family has been born. The recent arrival has been termed sulfabenzamine, and it differs from sulfanilamide and the other members of the family in having the amino group separated from the benzene ring by a methylene group. It has the distinction of being effective against bacteria in the presence of pus, and, therefore, lends itself to especially good use on the battlefield. The Winthrop Chemical Company has prepared this drug, which is a white, crystalline substance, under the name "Sulfamylon." The Germans have been using it for some time under the name Marfanil.

AJP

The war of legal rulings on vitamins goes merrily on, one of the latest States to have handed down a decision being New York. The Attorney General there has decided that vitamin preparations are really drugs, not foods. This follows similar edicts in New Jersey, Minnesota and Indiana. Thus it is that in these States concentrated vitamins that are official in the nation books of standards may be sold only in registered pharmacies by registered pharmacists.

AJP

The Council on Medical Education and Hospitals of the American Medical Association predicts a shortage of 15,000 physicians by 1948 if the present policy of student deferment is continued. There are those who see in the government policy a deliberate move to so impair medical service that popular demand for legislation such as the Murray-Wagner-Dingell Bill will grow more positive.

AJP

In view of the current serious outbreak of infantile paralysis, it is heartening to note that progress is being made in the scientific fight against the disease. Recently, in experimental work on mice, the virus that produces infantile paralysis was inactivated by exposure to ultra-violet rays, these rays destroying its disease-producing powers but not taking away its ability to produce antibodies. Vaccines prepared in this manner gave a high degree of protection to experimental animals.

If, sometime, you apply for a new phone or an extension line, and if you are refused, bear in mind that the Army and the Navy are using more than 350,000 of these scientifically developed instruments in the war effort. On one of our largest battleships alone there are more than 2,000 telephones.

AJP

Add to blood banks, eye banks, and other types of human-parts banks, a series of vein banks for storage of segments of blood vessels preserved by a quick-freezing process. Through a technique developed by Drs. Blakemore, Lord and Stefko, these reserve veins may be grafted into injured vessels, allowing for the passage of blood and eliminating to some degree the necessity for amputation.

AJP

A recent announcement from the Board for the Coordination of Malarial Studies of the National Research Council states in effect that Quinacrine Hydrochloride (Atabrine) is fully as effective as quinine in the suppression and treatment of malaria. It is probably even more effective and it has the additional advantage of not producing cinchonism. Totaquine, although as effective as quinine, is more likely to produce gastro-intestinal symptoms than either quinine or quinacrine.

AJP

The end of the war will not end our interest in antimalarial drugs. The population shift occasioned by overseas troops and war work is bound to result in malaria becoming an increasing problem even after the war. Mosquito control will also occupy the attention of our public health agencies and we may expect some radical changes in many sections of our mosquito-infested swamps.

AJP

The most rapid treatment ever conceived for a serious infection has been announced by the U. S. Public Health Service. Physicians of this group who treat men of the Coast Guard and Merchant Marine have obtained almost unbelievable results in the treatment of gonorrhea with penicillin. In one regimen of treatment only 7½ hours are required, five injections being given in this time. The percentage of cures was very high in spite of the fact that many of the cases were sulfonamide-fast and of long duration.

BOOK REVIEWS

The Standardization of Volumetric Solutions. By R. B. Bradstreet. VII + 151 pp. Chemical Publishing Co., Inc., 26 Court Street, Brooklyn, N. Y. Price: $3.75.

This book is intended as a reference work for industrial laboratory chemists. It includes a general discussion of volumetric analysis, calibration of apparatus, indicators, and standard substances. Methods for the preparation, standardization, and proper storage of all of the commonly used volumetric solutions and some of those less commonly used are given. Tables of data frequently required in standardization are also provided.

It is unfortunate that this book contains so many errors, both of commission and omission, and so many inaccuracies. These include incorrect chemical equations, incorrect equivalent weights, inadequate directions, and several glaring inconsistencies. After emphasizing the need for accuracy in standardization procedures, the directions in some cases would lead to inaccurate results. Another outstanding fault is the total disregard of the principle of significant figures.

In view of the exceptionally high price for a book of this size and composition, it is felt that so many faults are inexcusable.

L. A. Reber.

Plant Viruses and Virus Diseases. By F. C. Bawden. Second Edition, 294 pages including subject and author index, 48 illustrations. 1943. The Chronica Botanica Co., Faltham, Mass.; G. E. Stechert & Co., New York City. Price: $4.75.

A thoroughly revised edition of the original work, the type of which was lost during the invasion of The Netherlands. Much of the book has been entirely rewritten. The book summarizes modern

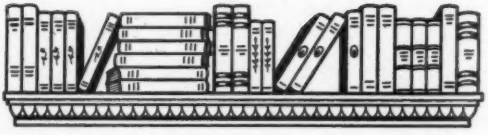
ideas and research techniques concerning virus diseases of plants. There are many useful tabulations, and the references accompanying each chapter are extensive.

Of especial interest to pharmacists and pharmacognocists are the references to the mosaic diseases of Hyoscyamus and Phytolacca, and the use of Phytolacca juice, pepsin, trypsin and papain as virus inhibitors to reduce the infectivity of these substances.

The chapters of the book include the following: Symptomatology; Transmission; Relationships between viruses and their insect vectors; Virus strains, mutation, and acquired immunity; Serological reactions of plant viruses; Methods of purification; Properties of purified virus preparations; Optical properties of purified virus preparations; Inactivation of viruses; The sizes of virus particles; Physiology of virus-diseased plants; The classification of viruses; The control of virus diseases; Origin and multiplication of viruses.

Plant Viruses and Virus Diseases is an outstanding compilation of the progress of work in this field.

E. H. MacLaughlin.



. . . put down one and carry two and multiply your shelf-space

IN OTHER WORDS, put down the wide, wasteful, old-fashioned bottle and pick up the narrow, book-shaped, Sharp & Dohme 'SPASAVER' bottles. They're *really* space-savers, as the name suggests. Flat on three sides and curved on the front like a book, they fit your shelves as books fit a book-shelf. Thirteen go into the space required for nine of the round or oval kind, giving you 44% more room.

'SPASAVER' bottles tone-up the appearance of your prescription-department, too. They are easy and comfortable to hold. They have "Pour-Out" lips which prevent dripping. A sturdy, plastic cap covers the entire lip and threads, keeping the pouring-surface clean. The label is lacquered and can be wiped clean with a damp cloth. Sharp & Dohme's tinctures, fluidextracts, and many other prescription-items are available in 'SPASAVER' bottles. Here is "room-service" . . . by **Sharp & Dohme, Philadelphia 1, Pa.**

SHARP & DOHME 'SPASAVER' BOTTLES

Effective, Convenient and Economical

THE effectiveness of Mercurochrome has been demonstrated by more than twenty years of extensive clinical use. For professional convenience Mercurochrome is supplied in four forms—Aqueous Solution in Applicator Bottles for the treatment of minor wounds, Surgical Solution for preoperative skin disinfection, Tablets and Powder from which solutions of any desired concentration may readily be prepared.

Mercurochrome

(H. W. & D. brand of merbromin. dibromoxymercurifluorescein-sodium)

is economical because stock solutions may be dispensed quickly and at low cost. Stock solutions keep indefinitely.

Mercurochrome is antiseptic and relatively non-irritating and non-toxic in wounds.

Complete literature will be furnished on request.

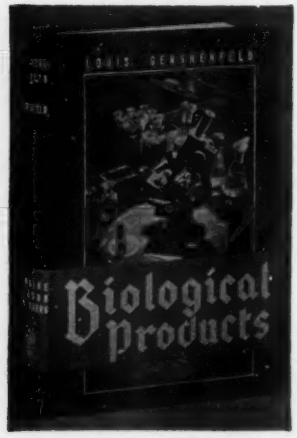
HYNSON, WESTCOTT & DUNNING, INC.

Baltimore, Maryland

"I WISH SOMEONE HAD GIVEN ME THIS BOOK WHEN I GRADUATED"

say pharmacists all over the nation.

This book supplies just the kind of information a pharmacist needs in order to have an intelligent understanding of biological products, their application in therapeutics and merchandising. If you want to boost your biological business get this book today.



BIOLOGICAL PRODUCTS

by Dr. Louis Gershenfeld, P.D., B.Sc., Ph.M.

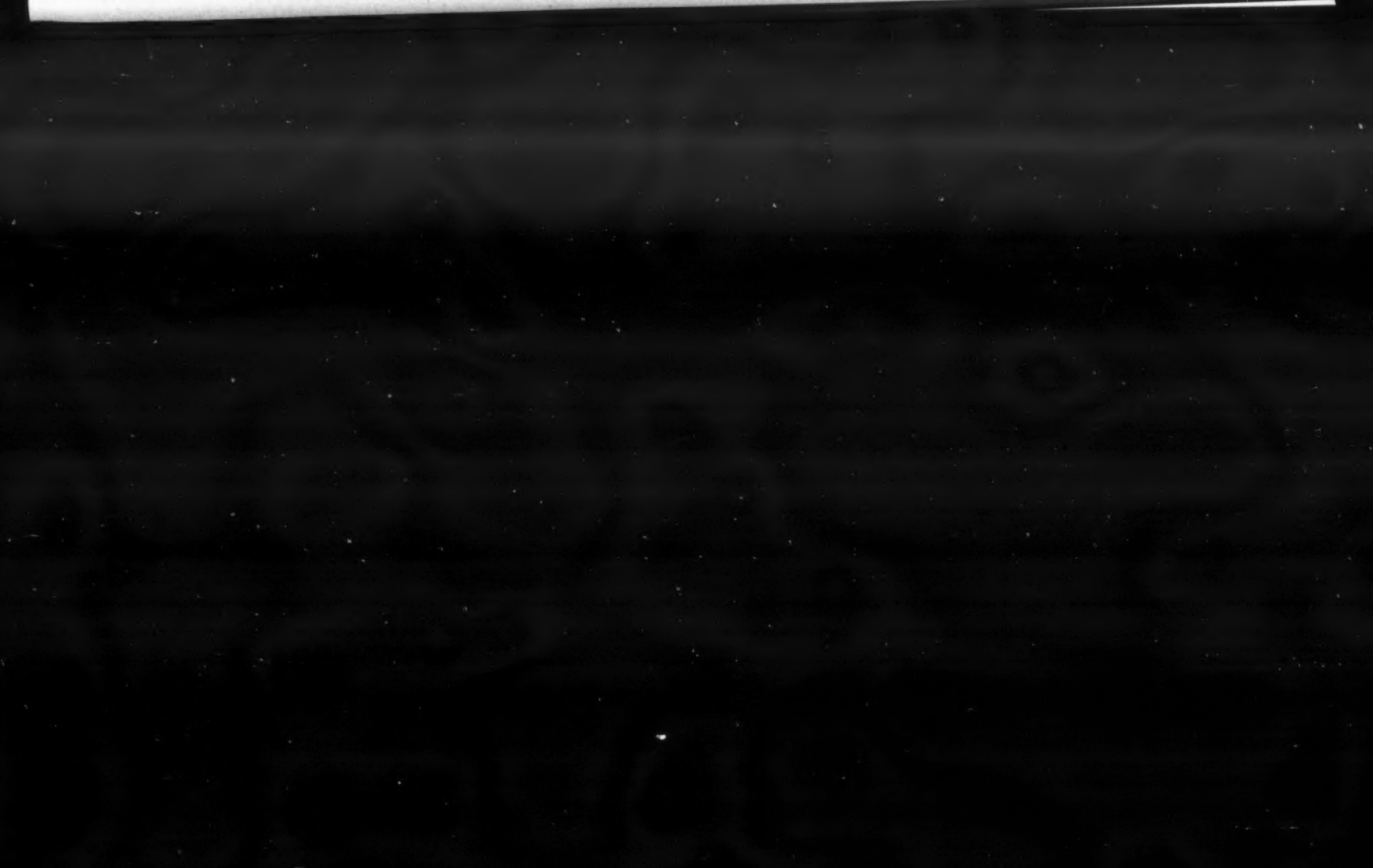
SOME OF THE SUBJECTS EMBRACED

Antitoxinum Diphthericum; Serum Sickness
Antitoxinum Tetanicum
Antitoxinum Scarlatinae Streptococcicum
Gangrene and Botulinus
Serum Antivenenosum, Venins, Venom, etc.
Antibacterial Serums
Serum Antimeningococcicum
Serum Antipneumococcicum
Antigens
Vaccinum Typhosum
Other Bacterial Vaccines, Plague, Cholera, etc.
Toxinum Diphthericum Detoxicatum, When to Immunize
Tetanus Toxoid
Tuberculinum Pristinum
Bacteriophage and Phagotherapy
Modified Viruses (Virus Vaccines)
Vaccinum Rabies
Yellow Fever
Poliomyelitis
Rickettsial Diseases of Man
Allergens and Sensitization Diseases

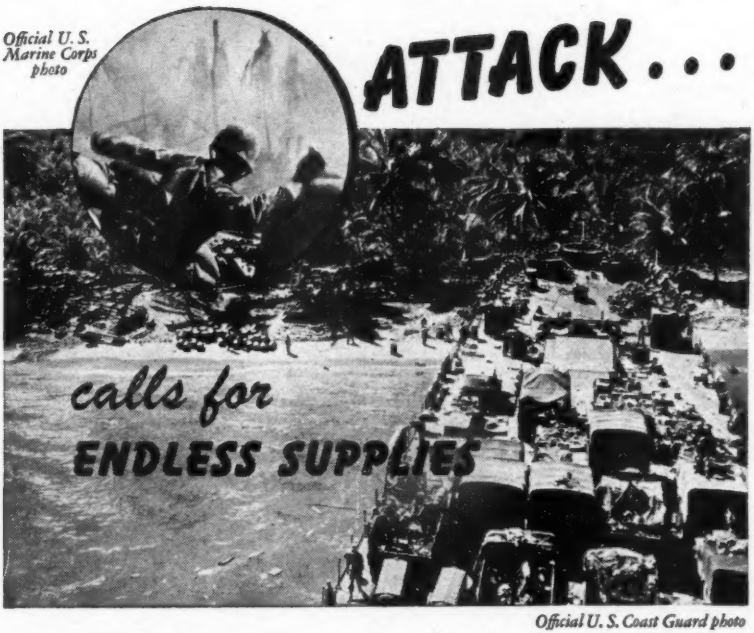
$4.00 plus 15c for postage and handling. ORDER NOW

ROMAINE PIERSON PUBLISHERS, INC.

67 WALL STREET NEW YORK 5, N. Y.



Official U. S. Marine Corps photo



Official U. S. Coast Guard photo

RETAILERS OF AMERICA, you can do a twofold job for the men who are fighting *to protect your future.*

By selling more War Bonds than before, you can help to save the lives of fighting men by making them the best supplied fighters in the world.

By selling more War Bonds than before, you can help to build up the purchasing power needed to provide jobs for our fighters in the post-war period.

Remember, our fighting men will have earned a full share in post-war America. Earn yours. Drive your War Bond Sales to an all-time high.

Don't ease up—*until the war is won!*

Back the Attack!

SELL MORE THAN BEFORE!

The Treasury Department acknowledges with appreciation the publication of this message by:

THE AMERICAN JOURNAL OF PHARMACY

This is an official U. S. Treasury advertisement prepared under the auspices of Treasury Department and War Advertising Council

Popular Science Talks

IN BOOK FORM

Interesting ● Readable ● Dependable

CONTENTS OF VOLUME VII

THE MODERN SUN CULT
ROMANCE OF BEVERAGES
SOIL AND SOD
TIME—WHAT KEEPS IT?
HOW MUCH DO YOU WEIGH?
THE COSMETIC URGE
SNAKES AND SNAKE PROTECTION
HEART BEAT AND BLOOD FLOW
CARBON OXIDE BROTHERS—MON AND DI
HISTORY AND MYSTERY OF PYROTECHNY
LITTLE DROPS OF WATER
EVOLUTION OF THE MOTION PICTURE
IODIZED SALT

For 21 consecutive years, the Philadelphia College of Pharmacy and Science has offered to the general public a series of Popular Science Talks delivered by members of the Faculty. Fourteen annual series have been compiled in separate books, and, of these, ten volumes are still available.

All of the books are paper-bound and are priced at $1 each, post-paid. They are in clear, readable type, amply illustrated, and make excellent reference works, for those who are interested in the general field of science. There are more than 100 subjects included in the ten volumes.

Send for a descriptive booklet

American Journal of Pharmacy

43rd Street, Kingsessing and Woodland Avenues

PHILADELPHIA 4, PENNSYLVANIA